REMARKS

Claims 1-17 were pending when the present Office Action was mailed November 23, 2007. In this response, claims 1, 5, 9, 14 and 15 have been amended and claim 11 has been canceled. New claims 27-40 have been added. No new matter has been added by way of these amendments. Accordingly, claims 1-10, 12-17 and 27-40 are currently pending.

In the November 23, 2007 Office Action, claims 1-17 were rejected and no claims have been allowed. More specifically, the status of the application in light of the Office Action is as follows:

Claims 1-17 are rejected under 35 U.S.C. 103(a) over any one of:

(1) Cejpek et al., Simplified Extraction and Cleanup Procedure for the Determination of PAHs in Fatty and Protein-Rich Matrices, (1995) ("Cejpek");
(2) Burdaspal et al., Determination of Polycyclic Aromatic Hydrocarbons in Pomace Olive Oil by Gel Permeation Chromatography and High Performance Liquid Chromatography, (2001) ("Burdaspal '01") (all references are made to translated copy provided by USPTO); or
(3) Burdaspal et al., Determination of Polycyclic Aromatic Hydrocarbons in Shellfish and Fish by Gel Permeation Chromatography and High Performance Liquid Chromatography, (2003) ("Burdaspal '03") (all references are made to translated copy provided by USPTO),

in combination with:

(4) Williams and Macrae, Non-Aqueous Size –Exclusion Chromatography Coupled On-Line to Reversed-Phase High-Performance Liquid Chromatography, (1989) ("Williams"); and
(5) Krishen and Tucker, Gel Permeation Chromatography of Low Molecular Weight Materials with High Efficiency Columns, (1977) ("Krishen").

I. Amendments

Claim 1 has been amended to clarify that that the eluting step includes eluting said sample with a water-miscible GPC eluting solvent, and that the method has a PAH detection limit of about 1 μg per kilogram of sample or less.

Claim 5 has been amended for clarification;

Claims 9 and 14 have been amended to correct antecedent basis;

Claim 15 has been amended to correctly depend from claim 5;

New claims 27-29 have been added and depend from claim 1; and

New independent claim 30 has been added and new claims 31-40 depend from claim 30.

No new matter has been added by way of these amendments.

II. Rejections Under 35 U.S.C. § 103

Claims 1-17 were rejected under 35 U.S.C. § 103(a) over any one of Cejpek, Burdaspal '01, or Burdaspal '03 in combination with Williams and Krishen. These rejections are respectfully traversed for the following reasons.

A. The Present Claims

Independent claim 1, as amended, is directed to a method for determining the level of at least one PAH in a sample selected from edible oils, edible fats, and components thereof, wherein the method has a PAH detection limit of about 1 μg per kilogram of sample or less. The method includes providing a sample in a first solvent in which each PAH is soluble. The method further includes applying the sample to a gel permeation chromatography (GPC) column and eluting the sample with a water-miscible GPC eluting solvent, effective to provide a fraction containing the PAH and which is substantially free of triglyceride and free fatty acid components of the sample. The method also includes injecting the fraction having a first volume, without isolation, into a GPC/HPLC interface. The method further includes adding a second volume of an aqueous solvent to the fraction, wherein the aqueous solvent includes a solvent in which each PAH has low solubility. The method also includes transferring the first and second volumes, without isolation, onto a reverse-phase high performance liquid chromatography (HPLC) column, and initially eluting the fraction with the solvent in which each PAH has low solubility. The method also includes separately eluting each PAH to be detected from the HPLC column with an HPLC eluting solvent, detecting at least one PAH, and determining the level of the at least one PAH in the sample.

B. The Applied Art

Cejpek discloses an extraction and cleanup procedure for determination of PAHs in fatty and protein-rich matrices. The procedure includes homogenization and ultra-sonication of solid food and fats in chloroform (approximately 150 ml total) to extract both PAHs and lipids. Following extraction, Cejpek teaches evaporation of chloroform from the sample on a heated rotary vacuum evaporator to about 3 ml total volume. (Cejpek, pg. 68-69, Procedure C). Following evaporation, Cejpek teaches cleanup of the sample on a GPC column, wherein the sample is loaded on the column and eluted with chloroform. After discarding a first 16.5 ml, a second 8.5 ml fraction (e.g., in chloroform) having the PAHs were collected and evaporated nearly to dryness. (Cejpek, pg. 69, Cleanup). After the chloroform was left exposed to evaporate spontaneously, the dry residue was resuspended in 0.2 ml acetonitrile. The resuspended fraction was analyzed by HPLC with fluorescence detection. (Cejpek, pg. 75-76, Identification and quantitation). Cejpek discloses use of acetonitrile/methanol/water mixtures in the HPLC determination process.

Burdaspal "01 discloses a method for determining the level of PAHs in pomace olive oil samples. Pomace olive oil samples were diluted with dichloromethane and separated on a gel permeation chromatography column. (Burdaspal '01, pg. 6, *Sample Preparation – Purification*). Fractions containing PAHs were collected and dried in a nitrogen flow and re-dissolved in 1 mL of acetonitrile. If more than one fraction was collected, these fractions were mixed, dried and re-dissolved together. (Burdaspal '01, pg. 7, *Purification*). The resuspended extract was then analyzed by high performance liquid chromatography (HPLC) with fluorescence detector and wavelength programming. (Burdaspal '01, pg. 4, *Objective – Foundation*). Burdaspal '01 reports a limit of determination of 0.57 μg/kg for each hydrocarbon. (Burdaspal '01, pg. 14, lines 20-21).

Burdaspal "03 discloses a method for determining the level of PAHs in mollusks and fish. Edible portions of mollusks or fish (e.g., 25 grams) were homogenized and diluted with dichloromethane. (Burdaspal '03, pg. 8-9, *Preparation of the Sample*). Following centrifugation, the supernatant (equivalent to 0.1 grams of sample) was separated on a gel permeation chromatography column and fractions corresponding to the elution of the PAHs

were collected in dichloromethane. (Burdaspal '03, pg. 8-9, *Preparation of the Sample – Purification*). The fractions were dried in an evaporator with a nitrogen flow and re-dissolved in 1 mL of acetonitrile. (Burdaspal '03, pg. 10, lines 1-3). The resuspended extract was then analyzed by high performance liquid chromatography (HPLC) with fluorescence detector and wavelength programming. (Burdaspal '03, pg. 10-11, *Liquid Chromatography*). Burdaspal '03 reports a limit of determination of 0.5 μg/kg for each hydrocarbon. (Burdaspal '03, pg. 18, lines 14-16).

Williams discloses a method for non-aqueous size-exclusion chromatography (SEC) coupled on-line to reversed-phase HPLC (RP-HPLC) for determination of contaminants in food at detection limits of about 50 ppb (0.5 mg/kg). Samples were dissolved in toluene, separated by SEC, and eluted in tetrahydrofuran. (Williams, pg. 318, *Size exclusion*). Williams teaches an interface between the SEC step and the RP-HPLC analysis, which includes eluting and isolating the required SEC fraction in tetrahydrofuran followed by continuous dilution of the sample in water prior to RP-HPLC gradient elution. (Williams, pg. 318-319, *Interface conditions – Reversed phase*). Williams discloses that the interface process can be automated.

Krishen teaches a gel permeation chromatography method using small-pore packing materials for increased resolution of low molecular weight analytes, including aromatic and cyclic hydrocarbons (Krishen, pg. 898, abstract; pg. 900, paragraph 3). Krishen reports using tetrahydrofuran to dissolve and elute samples. (Krishen, pg. 899, Procedure and Figure 2 legend).

C. <u>Analysis: Rejection of Claims 1-17 Based on Cejpek, Burdaspal '01 or Burdaspal '03 in Combination with Williams and Krishen</u>

The Office Action concedes that Cejpek, Burdaspal '01 and Burdaspal '03 (i.e., "the Primary References") all differ from the claims by not teaching interfacing of the gel permeation chromatography with the HPLC determination apparatus. The Office Action further acknowledges that the Primary References do not teach the use of tetrahydrofuran (THF) as the solvent in the gel permeation chromatography process (November 23, 2007 Office Action; pg. 3,

lines 8-10 and 27-29, and pg. 4, lines 12-15). The Examiner looks to Williams as allegedly supplying these elements.

Furthermore, the Examiner asserts that use of the column packing material and tetrahydrofuran as the elution solvent in the gel permeation separation step of Cejpek, Burdaspal "01 or Burdaspal '03, as taught by Williams and Krishen, would have been obvious to one of ordinary skill in the art at the time the invention was made, because (1) of its ability to separate triglycerides from other types of molecules as shown by Krishen and Williams, (2) its water miscibility as taught by Williams and (3) the rapidity of that process as taught by Krishen (November 23, 2007 Office Action, page 6, lines 6-15) . For the reasons explained below, however, the applicants respectfully disagree and stress that Williams and Krishen fail to cure the deficiencies of the Primary References in order to support a Section 103 rejection of claims 1-17.

According to the MPEP § 2143, "to establish a *prima facie* case of obviousness, three basic criteria must be met." The second criterion is that "there must be a reasonable expectation of success." The MPEP also clearly states that "[i]f a proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification." (MPEP § 2142.01; emphasis added.) Furthermore, "[i]f the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified then the teachings of the references are not sufficient to render the claims *prima facie* obvious." (Id.; emphasis added.)

Claim 1 is directed to a method for determining the level of one or more PAHs in an edible sample, wherein the method has a PAH detection limit of about 1 μg per kilogram of said sample or less. The method of claim 1 includes the features of injecting a GPC fraction having a first volume, without isolation, into a GPC/HPLC interface, adding a second volume of an aqueous solvent to the fraction. The aqueous solvent includes a solvent in which each PAH has low solubility. Claim 1 also includes transferring the first and second volumes, without isolation, onto a reverse-phase high performance liquid chromatography (HPLC) column.

As described in detail above, the Primary References disclose procedures for determination of PAHs in fatty and protein-rich matrices at detection levels required by foreign and domestic governing agencies. These procedures are not automated and require manually employed evaporation and/or drying steps following the GPC separation step. Williams teaches in-line coupling of SEC and HPLC separation and detecting steps for determining levels of water-insoluble contaminants in food. However, Williams' disclosure, which the Examiner wholly relies upon to teach automation of the separation and determination steps taught by the Primary References, teaches gross dilution of analyte SEC fractions. As a result, Williams reports, on page 324, detection limits of about 0.5 mg/kg (about 50 ppb) of analytes that are insoluble in water, and emphasizes in his report that this is a significant improvement over the detection limits (e.g., 200 mg/kg) provided by any of the methods of the prior art. (Williams, pg., 316, line 43 – pg. 317, line 23).

The Examiner asserts that it would have been obvious to one of ordinary skill in the art at the time the invention was made to perform any one of the methods taught in the Primary References in an automated manner as taught by Williams "because of the close relationship between size exclusion chromatography and gel permeation chromatography as taught by Williams" and because of "the benefits of automation as taught by Williams" (November 23, 2007 Office Action, page 6, lines 3-8).

Applicants respectfully disagree. First, the Examiner cannot merely pick and choose various elements from the prior art to come up with the claimed combination of elements without regard for the functionality of the resulting combination of elements. As discussed below, the resulting combination of elements would render the processes taught by the Primary References unsatisfactory and/or useless. Second, the combination of Cejpek, Burdaspal '01 or Burdaspal '03 with Williams teaches away from the features of the claimed invention.

1. The Cited Art Cannot Support a Section 103 Rejection Because the Resulting Process Would be Unsatisfactory and/or Useless

It is improper to combine Williams' automated interface used to transition fractions between SEC cleanup steps and contaminant determination on HPLC with the PAH cleanup and determination procedures of any of the Primary References because the resulting process would render these procedures unsatisfactory or useless for their intended purpose (i.e., determination of PAHs in fatty and protein-rich matrices at detection levels required by foreign or domestic governing agencies).

For example, the primary inventive aspect disclosed in Cejpek is the use of chloroform for a direct extraction of PAHs from meat products and other fatty products, followed by a simple cleanup and fractionation of PAHs and lipids with GPC using chloroform (in which PAHs have high solubility) as the mobile phase. Burdaspal '01 and Burdaspal '03 both utilize dichloromethane as the GPC mobile phase. Chloroform and dichloromethane are not good HPLC solvents because they are strong eluents on the HPLC column. In fact, one of ordinary skill in the art would recognize at the time of the invention that GPC solvents or mobile phases (e.g., chloroform) are not good HPLC solvents because they are strong eluents on the HPLC column. (e.g., Williams, pg. 317, *System development*, lines 7-12). To automate this transition, Williams teaches the necessity of at least a 1:4 dilution of the analyte fraction in water before being able to introduce the fraction to a HPLC column. (Williams, pg. 318-319, *Size exclusion – Interface conditions*). However, both chloroform and dichloromethane are immiscible in water and; therefore, based on the applicant's understanding, the dilution step of Williams would make Cejpek's, Burdaspal's '01 and/or Burdaspal's '03 procedures, as taught, inoperable for detection and analysis of PAHs following HPLC.

Accordingly, procedures disclosed in the Primary References include the non-automated evaporation/drying steps between GPC and HPLC process steps. Certainly, if any of the procedures of the Primary References, as directed, were combined with Williams, it appears that PAH losses would be significant due to non-mixing of the chloroform and/or dichloromethane solvents with water, and premature elution of the analytes due to the presence of these non-miscible solvents.

Relying solely on the teachings of Williams, the Examiner asserts that it would be obvious to use THF as the mobile phase in the procedures described in the Primary Reference because Williams discloses that chloroform (and toluene) are not usable with his process because they are immiscible with water. (November 23, 2007 Office Action, pg. 6, line 21 – pg. 7, line 4). Applicants respectfully disagree and direct the Examiner to the teachings of at least Cejpek. On page 72, Cejpek attests to employing a simple clean-up system "consisting of the widely used styrene-divinylbenzene copolymer Bio-Beads S-X3 with chloroform as a mobile phase (exclusion limit approx. 2000 Da.)" and explains that one of the reasons for applying this arrangement (presumably the use of chloroform with the widely used column packing material) was "good experience in its application for the separation of fat and PCBs." (Cejpek, pg. 72, lines 8-12). Moreover, Cejpek specifies that "more perfect conjugation of π-electrons in PAH molecules in comparison with PCBs gives better assumption for stronger retention of PAHs and consequently their better separation on styrene-divinylbenzene gel matrix with high conjugation of π-electrons." (Cejpek, pg. 72, lines 12-15). Cejpek's choice of solvent is deliberate because he found that not only pure 'size exclusion mechanism' controls the elution of PAHs, but also adsorption in the manner of π- π interactions with the gel which increases the retention of analytes. (Cejpek, pg. 72, line 25 – pg. 75, line 1). Cejpek references prior art methods using tetrahydrofuran as a mobile phase elution solvent; however, in his protocol, Cejpek chooses not use THF to elute PAHs, presumably because the less polar mobile phase enhances the adsorption effect, an effect that Cejpek notes can carry a higher risk of PAH degradation. (See Cejpek, pg. 75, lines 1-8; and pg. 78, lines 15-17).

The rejection of claim 1 over the proposed combination of the Primary References and Williams should accordingly be withdrawn for at least the reason that the process resulting from the combination would be inoperable for the intended purposes purported by any of the applied art.

2. <u>The Cited Art Cannot Support a Section 103 Rejection because the Combination of Cejpek, Burdaspal '01 or Burdaspal '03 and Williams Teaches Away from the Features of the Claimed Invention</u>

Claim 1 has been amended to clarify that the method has a PAH detection limit of about 1 µg per kilogram of sample or less. As a functional limitation, this amendment is sufficient to overcome an obviousness-type rejection. See, for example, *In re* Mills, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990]), and *In re* Land, 368 F.2d 866, 151 USPQ 621 (C.C.P.A. 1966), which states that Examiner's must afford patentable weight to functional limitations. In fact, the CCPA has specifically approved of this type of claim drafting by stating "there is nothing intrinsically wrong with the use of such a technique in drafting patent claims. Indeed, we have even recognized in the past the practical *necessity* for the use of functional language." (*In re* Swinehart, 439 F.2d 210, 169 USPQ 226 [C.C.P.A. 1971]; emphasis in original).

Furthermore, claim 1 is patentable under Section 103 because at the time the invention was made, one of ordinary skill in the art would not combine Williams' automatic SEC/HPLC interface technique requiring sample dilution in water with a process directed to achieving the highly desirable detection at low or sub-ppb levels of PAHs. Specifically, the processes of the Primary References and Williams do not suggest that elimination of the evaporation step would yield lower detection limits of PAHs to low or sub-ppb levels or even to FEDIOL (Féderation de l'Industrie d'Huilerie de la Communauté Européenne) approved detection limits.

It is imperative when establishing a *prima facie* case of obviousness to consider the skill level of the ordinary artisan in the relevant technology at the time the invention was made. (*Graham v. John Deere Co.*, 383 U.S. 1, 17-18, 148 U.S.P.Q. 459 (1966). Furthermore, the Supreme Court decision in *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727 (U.S. 2007), reaffirmed the holdings of *Graham*, and clarified "it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does." It is clear that, at the time of the present invention, both scientific pressures as well as the pressures applied by governing agencies were directing the skilled artisan to focus her efforts on <u>achieving lower detection limits</u>. Therefore,

one of ordinary skill in the art at the time the invention was made would not have been motivated to combine the processes of Cejpek, Burdaspal '01 or Burdaspal '03 with the column interface technology of Williams for determination and analysis of PAHs.

As disclosed in his section entitled *Interface Conditions*, Williams teaches that, for a 1 minute collection period, water is pumped into the "tee" union at the outlet of the SEC column at a constant 4.0 ml/min. rate, while the SEC THF mobile phase flows at a rate of 1.0 ml/min. Accordingly, the ratio of THF eluted fraction to water in William's systems is 1:4. (Williams, pg. 318-319, *Size exclusion – Interface conditions*). Following injection of the diluted SEC peak onto the reversed-phase column, 4 <u>additional</u> loop volumes of water (e.g., 20mL) are added to the reversed-phase column to "flush the sample onto the reverse phase. (Williams, pg. 317, *Apparatus and materials*, line 4, pg. 319, *Reversed phase*). Williams, in fact, discloses that "[t]he important parameter for solute reconcentration is its retention on the reversed phase column in a mobile phase consisting of water and THF" and that "the problem reduces to <u>calculation of the water-THF composition just permitting acceptable reconcentration</u>." (Williams, pg. 322, *Prediction of analysis conditions*, lines 7-10; emphasis added). While Williams recognizes 1) that dilution of the analyte fraction in THF with water is necessary for the automation steps he uses, and 2) that it is desirable to dilute the analyte fraction only the extent necessary, Williams also clearly teaches that fraction dilution at his reported ratio (e.g., 20% TFH), along with the flushing technique, is required for analytes having polarity equal or greater to phenol to achieve analyte reconcentration and loading on the head of the HPLC column. (Williams, pg. 322-323, *Prediction of analysis conditions*; and pg. 324, *Conclusions*).

Indeed, Williams concedes that "the extent of dilution with water required for the SEC peak to be retained on the reversed-phase column is an important system parameter...and the volume of water required for purging could be a constraint upon achievable sensitivity depending upon its purity." (Williams, pg 322, *Prediction of analysis conditions*, lines 1-5). As a result of his reported method, Williams reports, on page 324, detection limits of about 0.5 mg/kg (about 50 ppb) of analytes that are insoluble in water; however, he emphasizes in his report that this is a significant improvement over the detection limits (e.g., 200 mg/kg)

provided by any of the methods of the prior art. (Williams, pg., 316, line 43 – pg. 317, line 23). Critical here is the fact that the detection levels for food contaminants reported and optimized by Williams are significantly higher than the PAH detection levels resulting from the processes disclosed in the Primary References, as well as required by claim 1. Perhaps more importantly, the detection levels reported by Williams are significantly greater than FEDIOL approved or Ministerial Order status detection limits (see Application, pg. 2, lines 4-6; Burdaspal '01, pg. 2, lines 1-5; and Burdaspal '03, pg. 4, lines 20-25). In fact, the scientific objectives of Burdaspal '01 and Burdaspal '03 are to determine the level of PAHs by GPC/HPLC purification to achieve detection limits of 1.0 μg/kg and 0.5 μg/kg of sample, respectively (emphasis added). (Burdaspal '01, pg. 4, lines 2-6; and Burdaspal '03, pg. 5, lines 5-12).

The Examiner argues that "it is not clear what the detection level will be because Williams is not detecting PAH,...[h]owever there is an expectation that the detection level would be lower than that reported by either of Cejpek or Williams based on the fact that the Williams method eliminates a step of solvent removal that Cejpek clearly teaches as the primary step in which PAH are lost." (November 23, 2007 Office Action, page 7, lines 12-16). The Applicants respectfully disagree with this conclusion and stress that the Examiner has misapplied the data presented in the applied art, and that such a conclusion is scientifically unfounded. First, the analyte limits of detection reported by Williams are 2-3 orders of magnitude higher than the limits reported by the Primary References, regardless of the analyte in question. The difference in detection limits between the Primary References and Williams is simply not comparable and any ordinarily skilled artisan at the time of the invention looking to improve, or even maintain, the required detection limits for PAHs, would understand the teachings of Williams to be prohibitive to their primary objectives. Second, Williams acknowledges that the major limitation on sensitivity is the presence of co-extractives and that the detection limits he reports are constrained by 1) the considerable volume of water used as described above (pg. 322, *Prediction of analysis conditions*, lines 1-5), and 2) the limitations of mass loading on the clean-up (e.g., SEC) column (pg. 321, lines, 16-20), neither of which are curable by combining the methodologies of any of the Primary References with Williams. Williams discloses that the lipid sample requires initial dilution, and reports an upper limit of

sample loading "equivalent to a lipid injection of *ca.* 25 mg." (Williams, pg. 321, lines 20-27, and pg. 324, *Conclusions*).

The Examiner's assertion that an ordinarily skilled person in the art would remove the solvent evaporation or drying step included in the protocols of the Primary References, and replace that step with gross dilution taught by Williams, <u>simply because the loss of volatile PAHs detected by Cejpek was determined to occur at the noted evaporation step</u>, is improper. Applicants maintain that such a skilled person would not be motivated to replace the evaporation step with analyte fraction dilution, because he or she would be dissuaded from Williams' resulting detection levels. In fact, a person of ordinary skill in the art at the time the invention was made would not recognize that the automatic analytical in-line method of the present invention would yield desirable detection levels for PAHs, and therefore, <u>the results are surprising.</u>

The Applicants are mindful of the Examiner's position that there is allegedly "an expectation that the claimed detection limit of PAH would be reachable with the proposed combination" because "Williams teaches that it is possible to improve the process by modifying the method in ways such as lengthening the first column" and "recognizes that the detection limit can be improved and suggests at least one method of causing the improvement to happen." (November 23, 2007 Office Action, pg. 7, lines 16-21). However, Applicants respectfully submit that the techniques proposed by Williams to increase sensitivity (e.g., a longer SEC column, a flushable pre-column between the SEC and the RP-HPLC column, a third column following the RP-HPLC column, and post-column derivatisation) are labor- and/or reagent-intensive, may or may not be done in an automated manner, and/or require substantial additional time to complete the procedure – all of which teaches away from the objective of at least Cejpek (e.g., to provide a rapid method for PAH determination) and counters the market demand for simpler and cheaper processes for PAH determination.

Moreover, the Examiner's alleged expectation that the claimed detection limit of PAH would be reachable with the proposed combination, including one or more of the proposed procedural additions, is at most prophetic. The Examiner has provided no art or data in the field

to substantiate that any one of the proposed techniques proffered by Williams would yield PAH determination results on the order achieved by the processes of the Primary References or by the present invention. Krishen fails to cure the deficiencies of the Primary References because it does not teach elimination of the evaporation step, nor does it teach in-line coupling of GPC and HPLC process steps. Accordingly, the Section 103 rejection of claim 1 should be withdrawn.

In the decision in *KSR Int'l Co. v. Teleflex Inc.*, 127 S. Ct. 1727 (U.S. 2007), the Supreme Court confirmed that "the combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results," but "when the prior art teaches away from combining certain elements, discovery of a successful means of combining them is more likely to be nonobvious." As one skilled in the art would not be motivated by the cited references to modify or combine the references to produce the present invention, Applicants respectfully submit that the claimed invention is not obvious in view of the Primary References in combination with Williams and Krishen. Instead, Applicants submit that the novel relationship between the individual elements of claim 1 originates from the applicant's claimed invention and not the references themselves. Since hindsight is an improper rationale for a rejection under 35 U.S.C. § 103(a), and the Primary References in combination with Williams fails to establish a *prima facie* case of obviousness, withdrawal of the rejection of claim 1 is respectfully requested.

Claims 2-17 depend from claim 1. Therefore, the Section 103 rejection of these dependent claims should be withdrawn for the reasons discussed above and for the additional features of these claims.

III. New Claims

New claims 27-40 have been added. Claims 27-29 depend from otherwise allowable independent claim 1 and are fully supported in the specification as originally filed. For example, support for claims 27 and 28 can be found at page 15, lines 7-10, and page 21, lines 22-23; and support for claim 29 can be found at page 14, lines 16-19, and page 15, lines 7-10.

New claim 30 has been added and includes several features similar to independent claim 1. Additionally, claim 30 includes the features of injecting said fraction having a first volume and adding a second volume of an aqueous solvent to said fraction, wherein said second volume is approximately equal to said first volume. Support for this feature can be found throughout the originally filed specification and, for example, at page 15, lines 7-10.

New claims 31-40 depend from new independent claim 30 and are fully supported in the specification as filed. For example, For example, support for claim 31 can be found at page 18, lines 3-9; support for claim 32 can be found at page 16, lines 19-20; and support for claims 33-35 can be found at page 16, lines 21-23. Claims 36-38 are similar to new claims 27-29 and support can be found at the references section described above. Claim 39 is similar to originally filed claims 4, 5 and 15. Claim 40 can be found throughout the specification and, for example, at page 4, lines 24-27.

To the extent the 35 U.S.C. § 103(a) rejection is applied against new claims 30-40, Applicants respectfully request reconsideration. As Williams is clearly absent the features of claim 30 (e.g., adding a second volume of an aqueous solvent to the fraction wherein the second volume is approximately equal to the volume of the fraction (e.g., the first volume), the Examiner cannot establish a *prima facie* case of obviousness. Furthermore, by stressing the importance of his dilution parameters, Williams teaches away from the GPC/HPLC interface steps required by claim 30. Krishen does not teach or disclose these missing features, and therefore, fails to cure the deficiencies of the Primary References and Williams. Because there is lack of teaching in the art of methods for determining the level of at least one PAH in an automated manner, and because there is no disclosure or teaching in the art to restrict the dilution factor of analyte fractions in THF in a GPC/HPLC interface without isolation and/or an evaporation/drying step, the knowledge of a person having ordinary skill in the art at the time of the invention, would be insufficient to overcome the deficiencies of the applied art. Accordingly, a Section 103 rejection of new claims 30-40 should not be presented.

IV. Conclusion

In view of the foregoing, the claims pending in the application comply with the requirements of 35 U.S.C. § 112 and patentably define over the applied art. A Notice of Allowance is, therefore, respectfully requested. If the Examiner has any questions or believes a telephone conference would expedite prosecution of this application, the Examiner is encouraged to call the undersigned at (206) 359-8118.

Please charge any deficiencies or credit any overpayment to our Deposit Account No. 50-0665, under Order No. 334498004US1 from which the undersigned is authorized to draw.

Dated: May 16, 2008

Respectfully submitted,

By

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